

Amendments to the specification

On page 6, line 4 of the application, please replace the heading as follows:

List of Figures   Brief Description of the Drawings

Please amend the paragraph beginning on page 6, line 16 as follows:

Figures 2A-D shows specific staining for chitin obtained with fluorescein isothiocyanate (FITC) labeled MBP-CBD chitin probe.

Please amend the paragraph beginning on page 6, line 19 as follows:

Figure 3 shows sequences of chitin-binding domains of Chitinase A1 (Fig. 3-1), Chitovibrin (Fig. 3-23) and Chitinase VP1 (Fig. 3-2).

Please amend the paragraph beginning on page 7, line 12 to correct typographical errors and to add a sequence identifier as requested by the Examiner:

The term "Chitin Binding Domain" refers to a portion of a protein less than 70 amino acids, exemplified by the sequences in Figure 3 and having a consensus sequence:

W-5-Y-12-Y-5-H-7-P-S5-L (SEQ ID NO:4) where the numbers in the consensus sequence refer to the numbers of amino acids separating the conserved residues.

Please amend the paragraph beginning on page 13, line 6 as follows:

CBPs Chitin-binding domains can be either examined using standard fluorescent or light microscopy (*in situ* detection), or measured quantitatively using appropriate enzyme coupled assays like in ELISA (quantitation). *In situ* detection will allow the visualization of the morphology of chitin-containing structures and also the whole organisms when in combination with other existing cell staining method, such as DNA-staining DAPI, which allows the nuclei to be visualized. On the other hand, quantitation methods described above will allow the estimation of relative abundance of chitin content (thus the abundance of chitin-containing organisms), for diagnosis of the severity of infection or contamination by fungi. It is envisaged that FACS sorting can be used to analyze a large number of different chitin-containing organisms stained with CBD to determine which species are present in a sample and their relative abundance. This approach would be useful in cases where possible infections by multiple fungal species are suspected.

Please amend the paragraph beginning on page 8, line 10 to update the address of NEB as follows:

CBD can be constructed and expressed as a fusion protein to which a reporter is attached to form the diagnostic reagent. For example, MBP has been made as an in-frame fusion with CBD and the conjugate purified as described in the NEB catalog (New England Biolabs, Inc., ~~Beverly~~Ipswich, MA) (Example 1). Although the method in Example 1 specified the CBD from chitinase AI, the method is applicable for cloning any CBD desired for which a sequence is known.

Please amend the paragraph beginning on page 9, line 1 to update the address of NEB as follows:

CBD alone or a fusion protein containing a carrier protein and CBD can be used as an affinity tag for binding chitin-containing material. For example, the MBP-CBD can be cross-linked with a bifunctional reagent to a membrane, beads, filters or polymers known in the art. This will create a solid support that has high affinity for chitin. An example of this solid support is a CBD-coated magnetic bead (NEB catalog, New England Biolabs, Inc., ~~Beverly~~Ipswich, MA). The beads can be mixed with a chitin-containing solution and the magnetic particles to which the chitin has bound can then be pulled out of solution with a magnet. This simple enrichment step greatly aids in detection of chitin material in dilute solutions. The bound chitin can then be detected with a fluorescently labeled protein as previously described.

Please amend the paragraph beginning on page 9, line 22 to update the address of NEB as follows:

In a preferred embodiment, the chitin-detecting method utilizes the CBD from the C-terminal end of Chitinase A1 derived from *Bacillus circulans* WL-12 (NEB catalog, New England Biolabs, Inc., ~~Beverly~~Ipswich, MA). This CBD was synthesized as a fusion protein consisting of *E. coli* MBP and CBDcA1 (MBP-CBDcA1) in the Examples 2-7.

Please amend the paragraph beginning on page 19, line 23 to update the address of NEB as follows:

DNA sequence encoding CBDcA1 from plasmid pTXB1 (NEB catalog #E6900S, New England Biolabs, Inc., ~~Beverly~~Ipswich, MA) was inserted inframe into the SacI site of pMAL-p2X (NEB, cat#E8000S, New England Biolabs, Inc., ~~Beverly~~Ipswich, MA) at the C-terminal of MBP. The resulting construct was transformed into *E. coli* ER2566 (New England Biolabs, Inc., ~~Beverly~~Ipswich, MA) and used to express a fusion protein of MBP-CBD. The fusion proteins were purified using an amylose column (NEB catalog #E8021S, New England Biolabs, Inc., ~~Beverly~~Ipswich, MA) according to manufacturer's instructions. The purified protein was concentrated to yield app. 13.7 mg/ml and determined to be substantially pure as it migrated as a single band in SDS-PAGE.

Please amend the paragraph beginning on page 21, line 26 to update the address of NEB as follows:

Recombinant fusion proteins containing GFP as the fluorescent reporter were tested for CBD-based binding.  
(i) GFP was fused to the N-terminus of CBD (GFP-CBD), or (ii)

GFP was contained within a nuclear hormone receptor (Nhr)-CBD fusion protein (Nhr-GFP-CBD). In addition, both fusion proteins contain a spacer sequence intein between GFP and CBD (NEB 2002/2003 Catalog, p. 164, Product No. 6900S, New England Biolabs, Inc., ~~Beverly~~Ipswich, MA). GFP-CBD and Nhr-GFP-CBD were each over-expressed in *E. coli*. Total protein lysates were prepared from bacterial cells expressing each fusion protein and directly used as the staining reagent.